



Article

The Effects of Localized Plant–Soil–Microbe Interactions on Soil Nitrogen Cycle in Maize Rhizosphere Soil under Long-Term Fertilizers

Yanan Li ^{1,2}, Chengyu Wang ^{1,2}, Junnan Wu ³, Yumang Zhang ^{1,2,4}, Qi Li ^{1,2}, Shuxia Liu ^{1,2,*} and Yunhang Gao ^{5,*} 

¹ College of Resources and Environment, Jilin Agricultural University, Changchun 130118, China; liyanan2017217@163.com (Y.L.); wangchengyu2001@163.com (C.W.); zymcckj@sina.com (Y.Z.); dqil822@163.com (Q.L.)

² Key Laboratory of Soil Resource Sustainable Utilization for Jilin Province Commodity Grain Bases, Changchun 130118, China

³ Institute of Agricultural Resources and Environment Research, Jilin Academy of Agricultural Sciences, Changchun 130033, China; wujunnan426@163.com

⁴ College of Life Sciences, Changchun Sci-Tech University, Changchun 130600, China

⁵ College of Animal Science and Technology, Jilin Agricultural University, Changchun 130118, China

* Correspondence: liushuxia69@163.com (S.L.); gaoyunhang@163.com (Y.G.); Tel.: +86-15543133987 (S.L.); +86-13159752912 (Y.G.)

Abstract: Long-term fertilization can result in changes in the nitrogen (N) cycle in maize rhizosphere soil. However, there have been few reports on the impacts of plant–soil–microbe regulatory mechanisms on the N cycle in soil. In this study, soil samples were collected from a long-term experimental site located at Jilin Agricultural University, Changchun City, Jilin Province, Northeast China. We then analyzed the changes in the functional genes related to the N cycle, soil enzyme activity, and maize root exudates under long-term fertilizer application using metagenomics and liquid chromatography analysis. We aimed to investigate the response of the N cycle to long-term fertilizers, the interaction among plant, soil, and microbes, and the effect of the plant–soil–microbe system on the N cycle. Long-term fertilization had a significant effect on soil N contents, N₂O emissions, and enzyme activity related to the N cycle in maize rhizosphere soil. The functional genes of the N cycle were mainly enriched in the N degradation pathway in maize rhizosphere soil. N fertilizer application decreased the abundance of functional genes related to N fixation and degradation, denitrification, and assimilatory nitrate reduction (ANRA) and increased the abundance of functional genes participating in dissimilatory nitrate reduction (DNRA) and anaerobic ammonia oxidation/hydroxylamine oxidation. The soil environment was positively related to soil enzyme activity and negatively related to the microbial community composition and amino acids in root exudates. The contribution rate of microorganisms to the N cycle was the highest ($r^2 = 0.900$), followed by amino acids ($r^2 = 0.836$) and the soil environment ($r^2 = 0.832$). Therefore, we concluded that N fertilizer is the main factor limiting the soil N cycle and that microorganisms are the main factor regulating the N cycle in the plant–soil–microorganism system.

Keywords: N cycle; plant–soil–microbe system; enzyme activity; functional genes



Citation: Li, Y.; Wang, C.; Wu, J.; Zhang, Y.; Li, Q.; Liu, S.; Gao, Y. The Effects of Localized Plant–Soil–Microbe Interactions on Soil Nitrogen Cycle in Maize Rhizosphere Soil under Long-Term Fertilizers. *Agronomy* **2023**, *13*, 2114. <https://doi.org/10.3390/agronomy13082114>

Academic Editor: Nikolaos Monokrousos

Received: 15 July 2023

Revised: 4 August 2023

Accepted: 10 August 2023

Published: 12 August 2023



Copyright: © 2023 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

The nitrogen (N) cycle is the most basic ecological process in agricultural ecosystems. Soil N balance is very important for maintaining soil quality and crop growth and is directly related to the stability and productivity of agricultural ecosystems [1,2]. N is an important component controlling the stability and function of global ecosystems, and an increase in its content plays an important role in the stability of the soil nitrogen pool (reducing nitrogen leaching or gaseous loss) [3]. Fertilizers play an irreplaceable role in ensuring food security in China and have a significant impact on changes in soil nitrogen content and forms [4,5].

Studies have shown that long-term application of chemical fertilizers will increase the leaching of nitrate in soil and increase the emission of N_2O [6,7]. The application of organic fertilizers to soil can promote the transformation of N and increase the content of total nitrogen in the soil [8,9]. Therefore, it is very important to study the effect of long-term fertilization on the soil N cycle to improve the sustainable development of agriculture.

Soil microorganisms are an important part of maintaining soil function, and they are the basis for forming and maintaining soil fertility and soil health [10]. The classification and functional properties of soil microorganisms and the mechanism of microbial-mediated ecosystem processes are of great significance to the transformation of soil N pool-related functions [11,12]. The transformation of various forms of N includes fixation and degradation of N, nitrification, and denitrification, which are mainly driven by soil microorganisms [13]. Some functional microorganisms participate in organic nitrogen metabolism and nitrate reduction, such as ammonia-oxidizing bacteria, N-fixing bacteria, and nitrifying and denitrifying bacteria, which play an important role in the soil N cycle [14,15]. Soil microbial diversity decreases when a chemical fertilizer is applied, but the soil microbial community develops in a healthier direction when an organic fertilizer increases the ratio of fungi to bacteria [16–18]. In addition, the application of different nitrogen fertilizers may induce gene expression, metabolism, nitrogen uptake, and assimilation in plants and affect the enrichment of rhizosphere microorganisms and the abundance of nitrogen-cycling genes [19,20]. Although a large number of studies have shown that fertilization will affect the diversity and function of the soil microbial community [21–23], the regulation of soil microbial N under long-term fertilization needs further study.

Plants, soil, and microorganisms are a symbiotic system, and they interact with each other. Studies have shown that plant root exudates contribute to microbial growth, and microorganisms also secrete some compounds, such as acidic components, hydroxyl ions, phenolic compounds, and phosphatases [24,25]. These compounds can promote plant growth and resist biotic and abiotic stresses [13,24]. Plants select rhizosphere microbial communities through rhizosphere deposition, thus providing carbon and nitrogen compounds for microorganisms to grow [26]. Plants rely on nutrients provided by the soil to complete their growth and development, and in the process of plant growth, chemicals are released through the rhizosphere to improve the soil environment [27,28]. Studies have confirmed that most soil–plant–microorganism interactions occur in rhizosphere soil less than one millimeter away from the root surface; therefore, it is of great significance to explore the plant–soil–microorganism interactions in the rhizosphere soil environment in terms of soil fertility and crop yield [29,30]. In addition, the plant–soil–microorganism system acts on the soil N cycle [30]. Through rhizosphere deposition, plants influence the species of the rhizosphere microbial community, thus providing carbon and nitrogen compounds for the growth of microorganisms [26]. The interactions between plants and soil, such as material exchange and information transmission, cannot be separated from the participation of root exudates [31]. Root exudates also affect soil microbial diversity and function. Studies have shown that root exudates can affect microbial community diversity, promote soil nitrate reduction and denitrification, and affect the end products of denitrification [32–35]. Soil enzymes also play an important role in the plant–soil–microorganism system and have a significant impact on the N cycle. Past research has shown that nitrogen assimilation enzymes, ammonia assimilation enzymes, and biological nitrogenases can transform organic nitrogen into ammonium salts, which can be absorbed and utilized by plants [36]. However, the research on plant–soil–microorganism interaction under long-term fertilization is not comprehensive, and the effect of the plant–soil–microorganism system on the N cycle needs further study.

In this study, metagenomics sequencing and liquid chromatography technology were applied to quantify the N cycle (including soil N content, N_2O emission, and the functional genes related to the N cycle), maize root exudates, and soil enzyme activity under long-term fertilization, and to analyze the N cycle response to the plant–soil–microorganism system. We aimed to (i) explore the effects of long-term fertilizer application on the soil

N cycle process; (ii) analyze the interaction among plant, soil, and microorganisms; and (iii) examine the effect of the plant–soil–microorganism system on the N cycle under long-term fertilizers.

2. Materials and Methods

2.1. Experimental Design

Rhizosphere soil samples were collected from a long-term experimental site located at Jilin Agricultural University, Changchun City, Jilin Province, Northeast China, which has a semi-humid temperate continental climate (43°47'42" N, 123°20'45" E). The maize field experiment was established in 1984, with the maize seeds sown in April and all maize residues removed from the plot after harvest in October. The maize was rainfed without additional irrigation. The type of soil is black soil (Phaeozems). We collected the rhizosphere soil in polyethylene bags by shaking the roots until the non-adhering soil fell off in 2019. Soil samples were collected after the harvest period. Each plot was sampled at five points, and the samples were mixed into one sample for a total of eighteen test samples. Six treatments, including no fertilization (CK), N fertilization (N), compound chemical fertilization (NPK), maize straw return (S), the combination of phosphorus and potassium fertilizer with maize straw (PKS), and the combination of compound chemical fertilizer with maize straw (NPKS), were implemented with three replicates per treatment for this study. The chemical fertilizers were applied from 1984 to 2019. The number of fertilizer applications is listed in Table 1. After removing plant roots, the soil was screened by 2 mm and stored in a refrigerator at 4°C for determination of soil enzyme activity. Mature maize plants were selected, and the roots were removed and brought back to the laboratory for treatment, which was used to determine root exudates.

Table 1. Fertilizer dosage in experimental field.

Treatment	N Fertilizer kg/ha	P Fertilizer kg/ha	K Fertilizer kg/ha	Maize Straw kg/ha
CK	0	0	0	0
N	150	0	0	0
NPK	150	75	75	0
S	0	0	0	5000
PKS	0	75	75	5000
NPKS	150	75	75	5000

Note: the N fertilizer is urea (46%), the P fertilizer is heavy superphosphate (45%), and the K fertilizer is potassium sulfate (52%).

2.2. Determination Method

2.2.1. Method for Determination of Soil Physical and Chemical Properties, Enzyme Activity, and Root Exudates

Soil pH was measured by a pH meter. Total N (TN) was measured by the Kjeldahl method, and total phosphorus (TP) was measured by alkali fusion molybdenum antimony anti-spectrophotometry. Total potassium (TK) was measured by the NaOH melting-fire photometer method. Ammonium-N ($\text{NH}_4^+ - \text{N}$) and nitrate-N ($\text{NO}_3^- - \text{N}$) were measured by a continuous flow analyzer. Available phosphorus (AP) and available potassium (AK) were measured by the 0.5 mol/L NaHCO_3 method and NH_4CO_3 method, respectively. Readily oxidizable organic carbon (ROC) was determined by the potassium permanganate oxidation method, and soil organic carbon (SOC) and dissolved organic carbon (DOC) were determined by the TOC analyzer [37]. Determination of microbial biomass carbon (MBC) was performed by chloroform fumigation extraction [38]. The measured soil properties are listed in Appendix A, Table A1. Extraction was carried out according to the instructions of the soil enzyme activity kit, and soil enzyme activity, including N fixation enzymes, protease enzymes, nitrification enzymes, denitrification enzymes, and ureases enzymes, was determined by a microplate reader.

In determining root exudates, the retrieved plants were washed away from the soil on the root surface and cultured in 1000 mL of distilled water for 48 h at 25 °C. A rotary evaporator (100 mL to 4 mL) was concentrated on the root exudates, the amino acid and organic acid contents were determined by a liquid-phase analyzer, and the soluble sugar content was extracted by a reagent kit and determined by a microplate analyzer [39].

2.2.2. Metagenomics Sequencing, Contig Assembly, and Annotation

Microbial DNA was extracted according to the manufacturer's instructions, and metagenomic sequencing was conducted at Shanghai Personal Biotechnology Co., Ltd. (Shanghai, China), following the detailed steps listed in previous research [40]. The nonredundant contig set was obtained by using MMseqs 2 software. The (merged) contig sequence set was deredundant according to the similarity degree of 95% and the coverage degree of alignment area of 90%. Then, the contig sequence set was further filtered to remove nontarget fragment sequences and sequences with an actual depth of 0. Subsequently, contigs were used for the prediction of open reading frames (ORFs) using MetaGeneMark (<http://exon.gatech.edu/GeneMark/> accessed on 1 March 2021) and then translated into amino acid sequences. The nonredundant gene catalog was searched against the Kyoto Encyclopedia of Genes and Genomes (<http://www.genome.jp/kegg/> accessed on 15 March 2023) for functional annotation.

2.3. Data Analysis

We used analysis of variance (ANOVA) to analyze the differences among means in soil physical–chemical properties, followed by the least significant difference (LSD) test if the difference was significant, and Spearman's rank correlation coefficients to examine the associations between the soil enzyme activity and soil properties and bacteria. The influencing factors were divided into four groups, namely, plant variables (root exudates), soil environmental variables (soil physical and chemical properties), microbial variables (microbial diversity, microbial composition (Figure A1)), and soil enzyme activity. The key factors of each influencing factor were obtained by principal component analysis, and then the significant relationship among plants, soil, and microorganisms was analyzed by the key factors using Spearman's analysis and output by a pie network [41]. To analyze the influencing factors of the soil N cycle, redundancy analysis was used to select the factors that contributed the most to the functional genes of the N cycle in each group of variables, and then the contribution rate of each factor to the soil N cycle was calculated [42].

3. Results

3.1. Effects of Long-Term Fertilization on the Soil N Cycle

3.1.1. Effects of Long-Term Fertilization on Soil Nitrogen Content and N₂O Emissions

Long-term fertilization significantly increased the TN content in the soil, and the TN content in the straw treatment was significantly higher than that in the chemical fertilizer treatment. The TN content in the NPKS treatment was the highest, which was 0.34 g/kg higher than that in the CK treatment. The NH₄⁺–N content was also the highest under the NPKS treatment. The addition of straw increased the soil alkali-hydrolyzable nitrogen (ALN) content, and the NO₃[–]–N content in the treatment with the chemical fertilizer was significantly higher than that in the treatment with straw addition. Fertilization also affected the percentage of nitrogen content to TN in the soil, and the ALN content was lower than that of CK except in the NPKS treatment, while fertilizer application increased the proportion of NH₄⁺–N and NO₃[–]–N to TN. During the whole growth period of maize, the N₂O emissions were the highest at the large bell-mouth stage (long-term fertilization significantly affected the N₂O emissions at the large bell-mouth stage). The N₂O emissions of the N and NPK treatments were significantly higher than those of the other treatments, which were 236.93 and 230.45 mg·m^{–2}h^{–1} higher than those of CK, respectively (Figure 1).

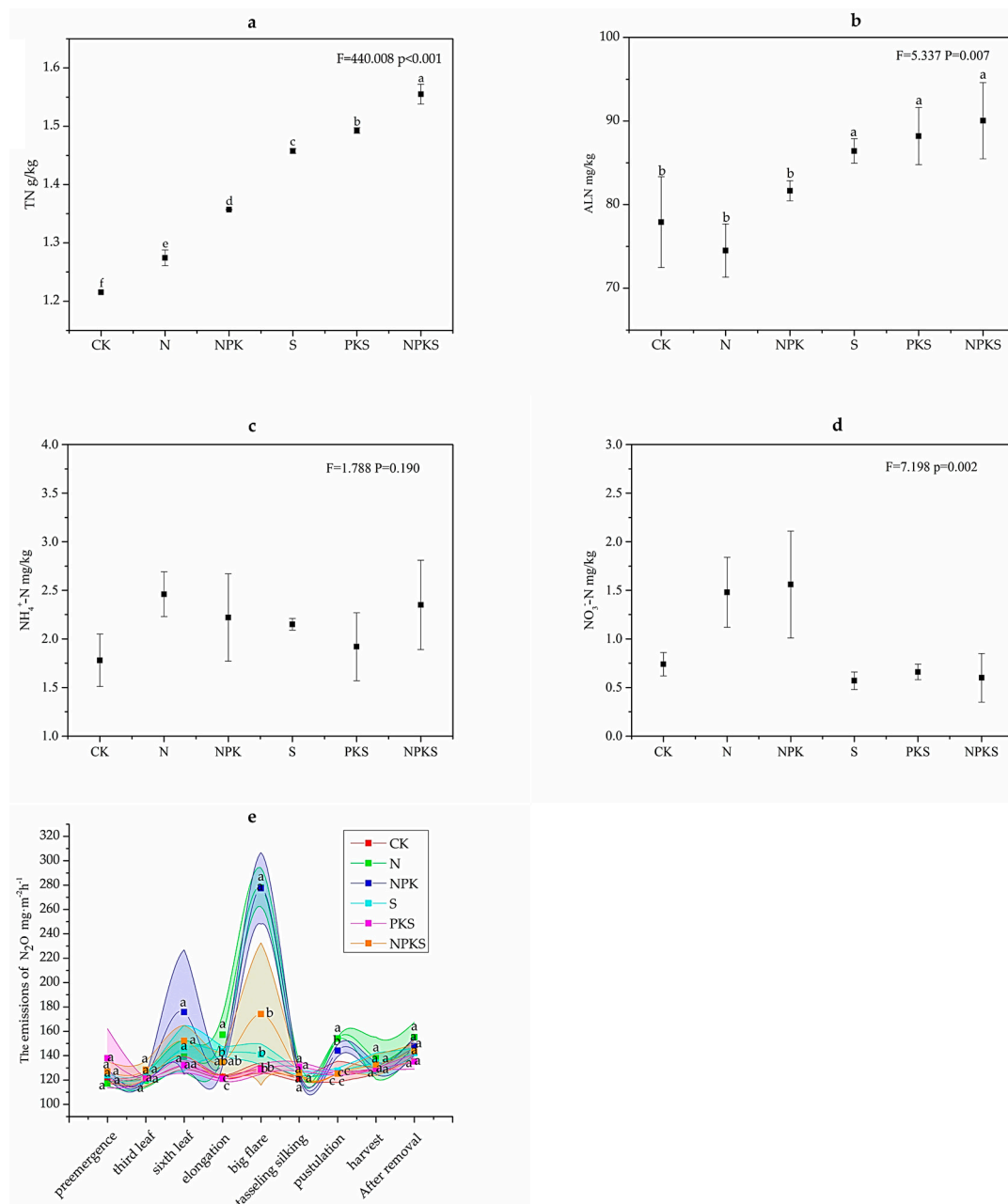


Figure 1. Effect of long-term fertilization on soil N content and N_2O emission. (a) TN (total nitrogen); (b) ALN (alkali-hydrolyzable nitrogen); (c) $\text{NH}_4^+\text{-N}$ (ammonium-N); (d) $\text{NO}_3^-\text{-N}$ (nitrate-N); (e) emission of N_2O . The lower case letters 'a', 'b', 'c', 'd', 'e', and 'f' indicate a significant difference ($p < 0.05$) among different samples for each treatment ($n = 18$) using the ANOVA method.

3.1.2. Effects of Long-Term Fertilization on Enzyme Activities Related to the N Cycle in Maize Rhizosphere Soil

The activities of N-related enzymes in the soil were analyzed. The results showed that the highest activity of protease was 0.30–1.78 U/g in the rhizosphere soil of maize, and its activity increased significantly under long-term fertilization. The highest activity of protease was under the NPKS treatment. Urease had the second-highest activity (0.63–1.18 U/g). Compared with the CK treatment, the treatment with straw significantly increased urease activity, while the treatment with N fertilizer significantly decreased urease activity. The activities of nitrification (20.89–43.11 IU/mg) and denitrification enzymes (26.43–37.17 IU/mg) were relatively low. Long-term fertilization significantly increased the

activities of nitrification enzymes and decreased the activities of denitrification enzymes (Table 2).

Table 2. Effect of long-term fertilization on soil enzyme activity related to N cycle.

	NITs U/g	NEA IU/mg	Nar IU/mg	Protease U/g	Urease U/g
CK	0.11 ± 0.00 b	20.89 ± 1.95 e	37.17 ± 3.37 a	0.30 ± 0.01 d	0.99 ± 0.01 c
N	0.11 ± 0.01 b	28.79 ± 0.75 c	31.85 ± 0.88 b	0.83 ± 0.17 c	0.83 ± 0.02 d
NPK	0.13 ± 0.00 a	25.12 ± 0.62 d	28.66 ± 0.27 c	1.47 ± 0.19 b	0.63 ± 0.00 e
S	0.13 ± 0.00 a	43.11 ± 4.59 a	32.80 ± 1.36 b	1.26 ± 0.02 b	1.18 ± 0.09 a
PKS	0.13 ± 0.00 a	28.58 ± 3.31 b	26.43 ± 0.37 c	1.25 ± 0.09 b	1.15 ± 0.01 a
NPKS	0.13 ± 0.00 a	35.94 ± 1.28 b	27.52 ± 2.14 c	1.78 ± 0.11 a	1.06 ± 0.02 b

Note: NITs: N fixation enzymes; NEA: nitrification enzyme; Nar: denitrification enzyme; Urease: urease enzyme; Protease: protease enzyme. The lower case letters 'a', 'b', 'c', 'd', and 'e' indicate a significant difference ($p < 0.05$) among different samples for each treatment ($n = 18$) using the ANOVA method.

3.1.3. Effect of Long-Term Fertilization on N Cycle Functional Genes in Rhizosphere Soil of Maize

Twenty-seven functional genes related to the N cycle in maize rhizosphere soil were analyzed. The results showed that these genes were involved in nitrogen fixation, nitrification, denitrification, the assimilative nitrate reduction pathway (ANRA), the dissimilatory nitrate reduction pathway (DNRA), anaerobic ammonia oxidation/hydroxylamine oxidation, and the N degradation pathway. The functional genes related to the N cycle were mainly concentrated in the N degradation pathway, and the relative abundance of genes in this pathway accounted for 27.36–32.20% of the N cycle. The N fixation pathway, denitrification pathway, and ANRA accounted for 23.95–25.99%, 17.79–23.85%, and 15.38–17.16% of the N cycle, respectively (Table 3).

Table 3. Effects of long-term fertilization on functional genes related to N cycle in maize rhizosphere soil.

	N Fixation	Denitrification	Nitrification	ANRA	DNRA	Anammox/ Hydroxylamine Oxidation	N Degradation
CK	25.99 ± 0.68 a	17.79 ± 1.00 b	1.26 ± 0.16 a	16.98 ± 0.63 ab	6.68 ± 0.51 b	0.04 ± 0.00 b	31.25 ± 0.80 a
N	23.95 ± 2.20 a	23.21 ± 3.52 a	1.04 ± 0.32 ab	15.63 ± 1.13 ab	8.73 ± 1.28 a	0.10 ± 0.03 a	27.36 ± 0.73 b
NPK	24.24 ± 1.20 a	23.85 ± 1.68 a	0.59 ± 0.21 c	15.38 ± 0.83 b	7.63 ± 0.66 ab	0.08 ± 0.01 a	28.24 ± 0.90 b
S	24.53 ± 1.07 a	17.71 ± 1.17 b	0.93 ± 0.17 bc	17.51 ± 0.99 a	7.10 ± 0.72 ab	0.02 ± 0.00 c	32.20 ± 0.66 a
PKS	24.47 ± 0.81 a	18.34 ± 1.38 b	1.09 ± 0.18 b	17.16 ± 0.79 a	6.99 ± 0.43 ab	0.03 ± 0.01 bc	31.93 ± 0.94 a
NPKS	24.47 ± 1.69 a	18.34 ± 2.32 b	1.09 ± 0.14 b	17.16 ± 0.05 ab	6.99 ± 0.86 ab	0.03 ± 0.01 bc	31.93 ± 1.06 a

Note: ANRA: assimilatory nitrate reduction; DNRA: dissimilatory nitrate reduction. The lower case letters 'a', 'b', and 'c' indicate a significant difference ($p < 0.05$) among different samples for each treatment ($n = 18$) using the ANOVA method.

Some functional genes involved in the N cycle changed significantly under long-term fertilization. Compared with that of CK, the abundance of the *nifA* gene decreased by 1.26, 1.52, and 0.21% under the N, NPK, and NPKS treatments, respectively, while the abundance of the *nifH* gene increased by 0.08, 0.08, and 0.02% under the N, NPK, and NPKS treatments, respectively. Long-term fertilization also had a significant effect on N decomposition ($p < 0.001$). Compared with that of the CK treatment, the abundance of functional genes related to N degradation decreased significantly under N fertilizer application but increased significantly after straw addition. The abundance of the *ureC* and *GDH2* genes was the highest under the S treatment, at 9.21% and 20.97%, respectively. The abundance of the *ureC* gene was the lowest under the NPK treatment, which was 1.83% lower than that under the CK treatment. The abundance of the *GDH2* gene was the lowest under the N treatment, which was 3.09% lower than that under the CK treatment. The nitrification functional genes *amoA*, *amoB*, and *amoC* decreased significantly under long-term fertilization, especially under the N and NPK treatments. The response of denitrification functional genes to fertilization was opposite to that of nitrification functional

genes. Denitrification functional genes had the lowest abundance in the CK treatments, while the N and NPK treatments significantly increased the abundance of the *narG*, *narH*, *nirK*, *narB*, and *nosZ* genes. The abundance of the functional gene *nrfA* related to DNRA in the N and NPK treatments was significantly higher than that in the other treatments, while the abundance of the functional gene *nasD* related to ANRA in the N and NPK treatments was significantly lower than that in the other treatments. In addition, the N and NPK treatments also significantly increased the abundance of the functional genes *hao*/*hzo* related to anaerobic ammonia oxidation/hydroxylamine oxidation (Figure 2).

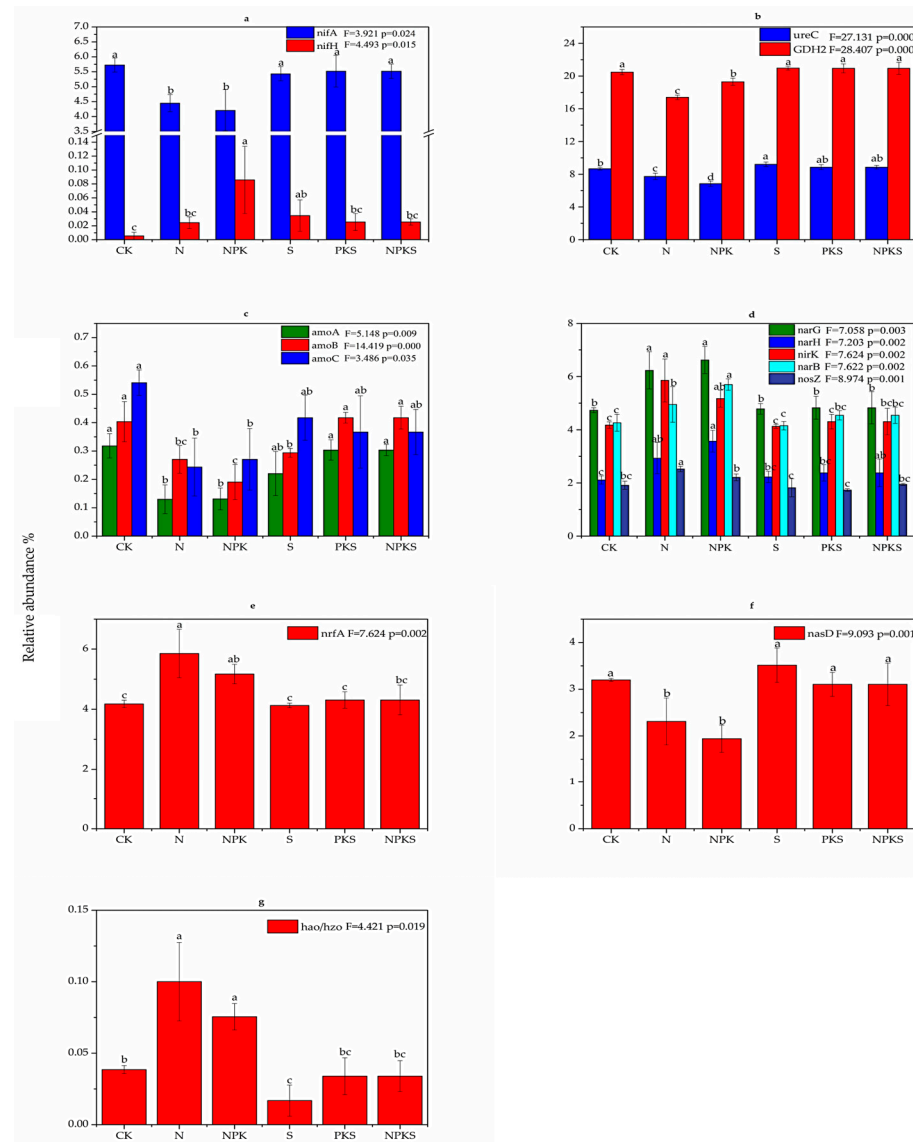


Figure 2. Relative abundance of functional genes participating in the N cycle (the significance test at $p < 0.05$ level) in maize rhizosphere soils under different fertilizer applications. (a) N fixation (*nifA*, *nifH*); (b) N derogation (*ureC*, *GDH2*); (c) nitrification (*amoA*, *amoB*, and *amoC*); (d) denitrification (*narG*, *narH*, *nirK*, *narB*, and *nosZ*); (e) DNRA (*nrfA*); (f) ANRA (*nasD*); (g) anammox/hydroxylamine oxidation (*hao*/*hzo*). The lower case letters ‘a’, ‘b’, and ‘c’ indicate a significant difference ($p < 0.05$) among different samples for each treatment ($n = 18$) using the ANOVA method.

3.2. Effect of Long-Term Fertilization on Root Exudates of Maize

Thirteen kinds of amino acids were found in the root exudates of maize, among which the tyrosine content was the highest, accounting for 50.41–91.10% of the total amino acids, and its content was the highest under the S treatment, which was 11.42 $\mu\text{g/g}$.

Long-term fertilization significantly reduced the histidine content, especially in the S and PKS treatments, which was lower than 0.31 µg/g. The methionine content in the N, NPK, and NPKS treatments was significantly higher than that in the S and PKS treatments. In addition, the contents of serine, threonine, and proline decreased significantly under long-term fertilization ($p < 0.05$) (Figure 3).

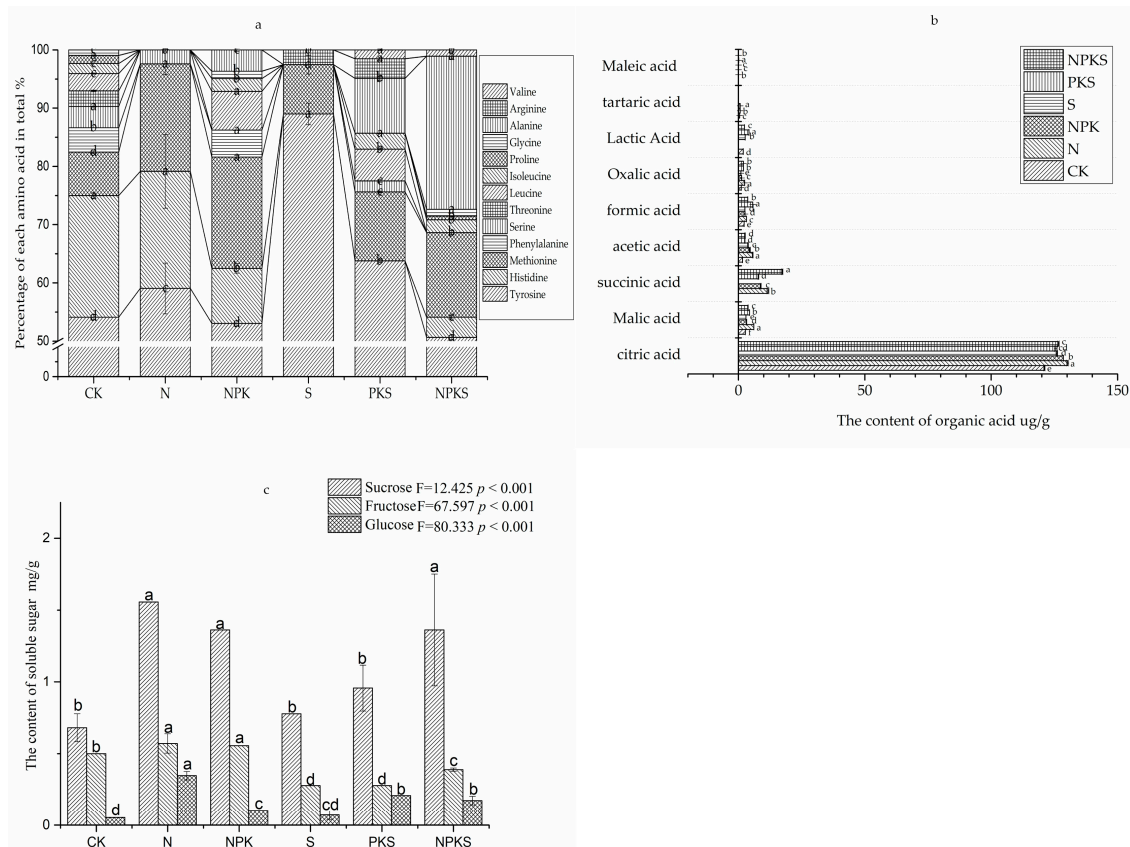


Figure 3. Effect of long-term fertilization on maize root exudates. (a) amino acid; (b) organic acid; (c) sugar. The lower case letters ‘a’, ‘b’, ‘c’, and ‘d’ indicate a significant difference ($p < 0.05$) among different samples for each treatment ($n = 18$) using the ANOVA method.

Nine organic acids were found in the root exudates of maize in this experiment, among which citric acid was the highest, accounting for 79.77–92.32% of the total organic acids. Long-term fertilization increased the citric acid content, especially in the treatment containing N fertilizer. The content of succinic acid increased significantly in the NPKS treatment. Long-term fertilization increased the content of formic acid and acetic acid, while the content of lactic acid decreased significantly under the N and NPK treatments and increased significantly under the S, PKS, and NPKS treatments ($p < 0.05$) (Figure 3).

The sugar content in the root exudates was sucrose > fructose > glucose, and the content of the three sugars changed significantly under long-term fertilization ($p < 0.001$). N, NPK, and NPKS significantly increased the sucrose content; compared with that of CK, the sucrose content increased by 0.97, 0.78, and 0.78 mg/kg, respectively. The fructose content also increased significantly under the N and NPK treatments but decreased significantly under the S, PKS, and NPKS treatments. The glucose content increased significantly under long-term fertilization (Figure 3).

3.3. Effect of Plant–Soil–Microorganism Interaction on the N Cycle

The principal component analysis was used to determine the principal component factors in each variable. There are four principal component factors in the soil’s physical and chemical properties: SOC, DOC, ROC, and TN. There are two main components in

soil enzyme activity, namely, nitrogenases and proteases. The Simpson index was the main factor in the microbial community diversity, and Elusimicrobia, Nitrospirae, and Firmicutes were the main factors in the community composition. A principal component analysis of sugar, organic acid, and amino acid in plant variables was carried out, and the main factors of phenylalanine, threonine, histidine, proline, arginine, citric acid, oxalic acid, and sucrose were finally determined (Table A3).

From the relationship diagram of significant correlations among plants, soil, and microorganisms, it can be seen that there is an extremely complex correlation among plants, soil, and microorganisms. There was a negative interaction between the soil environmental variables and the microbial community composition, and there was a significant negative correlation between DOC, ROC, TN, and SOC and Elusimicrobia and Nitrospirae ($p < 0.001$). There was a positive interaction between the soil environmental variables and soil enzyme activities, and there was a significant positive correlation between NITs and proteases and soil DOC, ROC, TN, and SOC ($p < 0.05$). Phenylalanine, threonine, and histidine in maize root exudates were negatively correlated with the soil environmental variables, especially with TN ($p < 0.05$). In addition, there was a significant negative correlation between DOC and threonine, histidine, and citric acid. Microbial diversity was significantly negatively correlated with the levels of sucrose and oxalic acid in plant root exudates, while it was highly significantly positively correlated with the levels of citric acid. There was a highly significant positive correlation between Elusimicrobia and Nitrospirae and histidine. There was a significant negative correlation between threonine and NITs and proteases (Figure 4, Table A4).

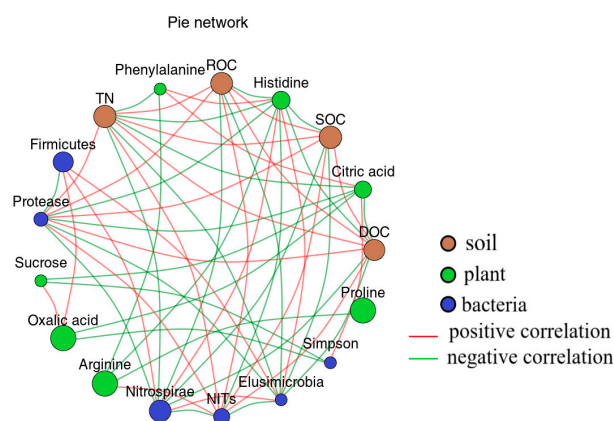


Figure 4. Interaction among plant, soil, and microorganisms.

The soil's physical and chemical properties, soil enzyme activity, root exudates, microbial diversity, and community composition have significant effects on the soil N cycle. The results showed that SOC in soil components; the Shannon index and Actinobacteria in microbial components; and urine enzymes, methionine, lactic acid, and sucrose in root exudates had the highest influence on the soil N cycle functional genes (Table A5). The soil environmental variables, microbial variables, and root exudates also had significant effects on soil N cycling ($p = 0.001$). Among them, the contribution rate of microorganisms was the highest, the r^2 of community composition and the N cycle was 0.900, and the r^2 of diversity and N-cycle genes was 0.841. The contribution rate of soil environmental variables to the N cycle was 0.832. The lowest contribution rate of soil enzyme activity was 0.640. The contribution rate of root exudates to the N cycle was amino acid > organic acid > sugar (Figure 5, Table A6).

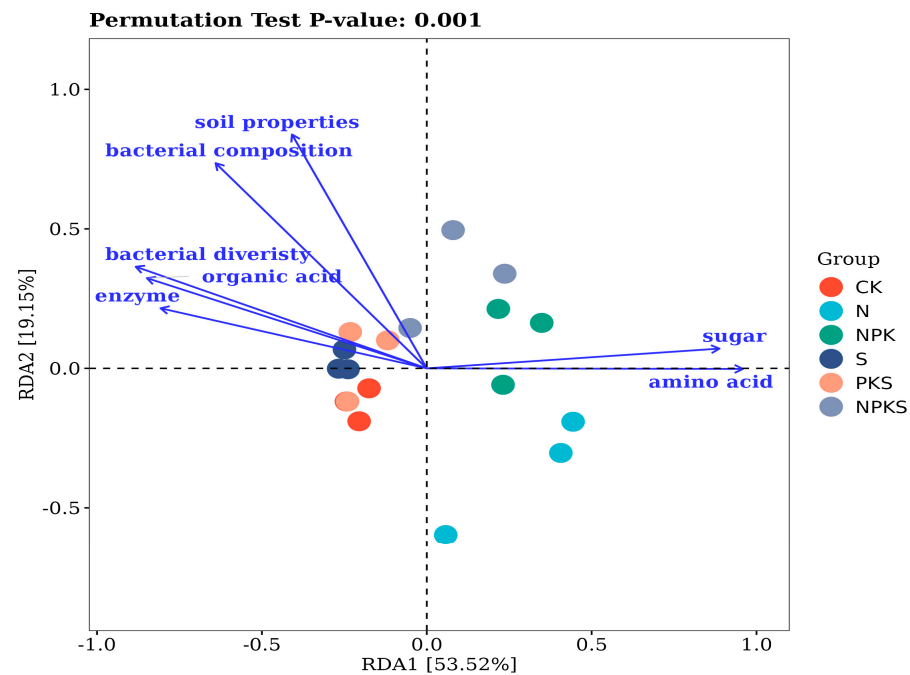


Figure 5. Contribution rate of each component to soil N cycle.

4. Discussion

4.1. Effects of Long-Term Fertilization on the Soil N Cycle

Fertilization plays a very important role in the soil N cycle [43]. The addition of fertilizers can increase the TN content within the soil, and the addition of straw will promote this effect. This is due to the application of chemical fertilizers, which increase the source of N in the soil. Additionally, the decomposition of straw releases the N contained in the straw into the soil, thus improving its fertility [44]. In this experiment, the N and NPK treatments significantly increased the $\text{NO}_3^- - \text{N}$ content and promoted the $\text{NH}_4^+ - \text{N}$ and $\text{NO}_3^- - \text{N}$ percentages to TN, but fertilization reduced the ALN/TN percentage (Figure 1, Table A2). This result is the same as that of Sun Ruijuan et al. [45]. N_2O emissions reached their highest value (Figure 1) at the big bell-mouth stage of maize, which may be due to the enhanced respiration ability of roots to grow in the big bell-mouth stage of maize. Furthermore, the soil temperature at this stage was suitable for the growth and development of microorganisms, which promoted their respiration, thus making N_2O emissions significantly higher than those at other growth stages [46,47].

Soil enzyme activity plays an important role in soil nutrient cycling and is a good index to measure the decomposition of soil organic matter [48,49]. Soil enzyme activity is very sensitive to environmental changes. Past research has noted that fertilization has a significant impact on soil enzyme activity, and the application of organic fertilizer can improve soil enzyme activity [50]. This study also reached a similar conclusion. The addition of straw promoted the activities of protease and urease. The activity of protease was highest in maize rhizosphere soil, which may be related to the increase in soil carbon and nitrogen content by long-term fertilization. Through correlation analysis, it was found that the activity of protease was significantly correlated with the soil TN, SOC, DOC, and ROC (Table 2, Figure A2). The addition of straw promoted an increase in soil nutrient content and nitrogenase activity, especially in the treatment with straw addition. In addition, the long-term application of chemical fertilizer decreased the activity of urease, which may be related to the change in soil microbial community diversity [51,52]. The long-term application of chemical fertilizer in this experiment decreased the relative abundance of Actinobacteria and Candidatus Tectomicrobia, which were significantly positively correlated with soil urease. Overall, long-term chemical fertilizer application also increased the relative abundance of Verrucomicrobia, Gemmatimonadetes, Armatimonadetes, and

Elusimicrobia, which were significantly negatively correlated with soil urease activity, thus reducing urease activity (Figures A1 and A2). The nitrifying enzyme activity increased significantly and the denitrifying enzyme activity decreased significantly under long-term fertilization, which may be related to the change in soil nutrient content [53]. SOC, DOC, and TN were positively correlated with the nitrifying enzyme activity and negatively correlated with the denitrifying enzyme activity (Figure A2).

The N cycle is an important biogeochemical cycle that is almost completely completed by the redox reactions of microorganisms [54,55]. There are many factors affecting soil N cycling, among which fertilization plays a very important role [43]. Because black soil has high fertility and can transform inherent soil nitrogen into $\text{NH}_4^+ - \text{N}$ [56], in this study, the proportion of the N degradation pathway in the N cycle was the highest, at 27.36–32.20%. In addition, the *ureC* and *GDH2* genes decreased significantly under N fertilizer application but increased significantly under straw application. This may be due to the decrease in soil pH caused by the long-term application of chemical fertilizer, which leads to a decrease in microbial diversity and a decrease in the ability of microbial decomposition of the soil's intrinsic N [19]. Moreover, the straw return increased the nutrients needed for microbial activity, increased microbial diversity, and then promoted the decomposition of the soil's inherent N [57]. Five nitrogen-fixing genes (*nifA*, *nifS*, *nifD*, *nifH*, and *nifK*) were identified in this study. Among them, the relative abundance of *nifS* was the highest, accounting for 18.87–20.21% of the N cycle, but long-term fertilization had no significant effect on its abundance. The gene abundance of *nifA* and *nifH* is strongly affected by N fertilizers. Some studies have shown that soil organic matter is the key factor driving nitrogen fixation in *nifH* [58]. In this study, the SOC content in the N and NPK treatments was lower than that in the other treatments, which led to a decrease in nitrogen fixation gene abundance. In addition, the functional genes in different N transformation processes changed significantly under long-term fertilization, which indicated that fertilization changed the transformation among various forms of N. Numerous studies have shown that soil nitrification and denitrification are important processes in the biogeochemical cycle of farmland soil and have a strong response to nitrogen fertilizers [59–61]. In this experiment, the application of nitrogen fertilizer reduced soil microbial nitrification and ANRA and promoted denitrification and DNRA. *amoA* and *amoB* abundance decreased significantly in the N, NPK, and S treatments, but there was no significant difference between PKS and NPKS. *amoC* abundance was the highest in the CK treatment. There are seven functional genes involved in denitrification in maize rhizosphere soil, among which the abundance of *narG*, *narH*, *narB*, *nirK*, and *norZ* significantly increased under long-term N fertilizer application, which promoted the emission of NO, N_2O , and N_2 . However, the addition of straw had no significant effect on it. Although the abundance of *hao/hzo* genes involved in anaerobic ammonification/hydroxylamine oxidation is significantly lower than that of other functional genes, it is actually a chemical autotrophic pathway without organic carbon, so it also has a crucial impact on soil nitrogen loss [62]. The abundance of *hao/hzo* decreased significantly under the N and NPK treatments but increased significantly under the S treatment. ANRA and DNRA can transform N from the highest oxidized state ($\text{NO}_3^- - \text{N}$) to the lowest reduced state ($\text{NH}_4^+ - \text{N}$), obtain energy from organic carbon, and enhance soil-available N retention for crop uptake and utilization [63]. In this study, the N and NPK treatments decreased *nasD* abundance and increased *nrfA* abundance. The *nasD* gene was also affected by straw, while the S treatment increased its abundance. In addition, compared with the application of chemical fertilizer, straw return increased the abundance of N fixation and decomposition genes, promoted nitrification, and inhibited denitrification. The above results indicate that straw return to the field may reduce the loss of N in black soil, and the application of a N fertilizer will increase the emission of N_2O .

4.2. Role of Root Exudates in the N Cycle of Maize

Tyrosine and phenylalanine aromatic exudates were detected in maize root exudates, and the content of tyrosine was significantly increased in the treatment without N fertilizer,

which also indicated that straw return could improve the ability of plants to produce pigments, defense compounds, and lignin (cell wall components) [64]. In this study, the methionine content increased significantly under long-term fertilization, especially in the treatment containing N fertilizer, and N fertilizer also increased the methionine content. All the above results indicate that N fertilizer was the main factor regulating amino acid secretion from maize roots. Citric acid had the highest content of organic acid in the root exudates of maize, which was significantly increased under long-term fertilization, especially in the treatment containing N fertilizer. A previous study has shown that there is a significant correlation between citric acid content and soil pH [65]. The increase in citric acid content promoted a decrease in soil pH (Table A1). In this study, the total amount of organic acids increased significantly under long-term fertilization, resulting in soil acidification. In addition, formic acid and acetic acid increased significantly under long-term fertilization, which was similar to the research results of Hu et al. [62]. The addition of straw promoted the lactic acid content secreted by corn and accelerated the degradation of corn straw. The content of sucrose in the root exudates of maize is the highest, which may be because sucrose is the main product of plant photosynthesis and carbohydrate transport is mainly in the form of sucrose in plants [66]. The contents of sucrose, fructose, and grape increased significantly after the long-term application of N fertilizer, which may be due to the increase in photosynthesis of plants and the increase in sugar content transmitted to the soil [67]. The addition of straw promoted glucose content and decreased fructose content, which indicated that the content of sucrose converted into glucose increased after the addition of straw.

4.3. Role of Plant–Soil–Microorganism Systems in N Cycle

Given that plants, soil, and microbes are tightly linked in the N cycle, long-term fertilizer application changes in the soil's physicochemical properties can be regarded as potential mechanisms driving the dynamics of plants, soil, and microbes and their interaction [41]. In this paper, through the correlation analysis of the main components in plants, soil, and microorganisms, similar conclusions are drawn. In maize root soil, there was a negative interaction between the soil environment and the microbial community composition, and there was a significant negative correlation between soil SOC, DOC, ROC, and TN and microbial Elusimicrobia and Nitrospirae. That is, under long-term fertilization, the contents of soil carbon and nitrogen nutrients increased significantly, which reduced the stability of the microbial community. Studies have shown that changes in the soil environment also change soil enzyme activity [68,69]. In this paper, soil enzyme activity was significantly related to the soil environment. Fertilization not only enhances soil nutrient content but also enhances the substrate needed by microbial activity, which promotes the enhancement of enzyme activity related to the N cycle. Previous studies have shown that root exudates can affect the microbial community and diversity [70,71]. In addition, Maurer et al. [72] confirmed that root secretion is significantly related to soil denitrification. It was found that amino acids and organic acids in maize root exudates negatively interacted with the soil environment, which showed that DOC, ROC, SOC, and TN in the soil were significantly negatively correlated with histidine under long-term fertilization conditions. In addition, TN was negatively correlated with phenylalanine, threonine, histidine, and citric acid.

The correlation between plants, soil, and microorganisms has a significant impact on the N cycle. The soil nitrogen cycle can be controlled by both biotic and abiotic factors. Studies have shown that soil nitrification and denitrification are affected by soil pH, soil water content, and environmental factors [73,74]. The changes in root exudates, enzymes, and microorganisms had significant effects on the soil N content [75]. The contribution rate of each component to the N cycle was obtained by redundancy analysis, and it was found that the contribution rate of microorganisms was the highest (composition $r^2 = 0.900$, diversity $r^2 = 0.841$), which may be because each reaction of the N cycle was driven by microorganisms; for example, nitrogen fixation was dominated by AOA and AOB, and

nitrification and denitrification were dominated by nitrifying bacteria and denitrifying bacteria, so microorganisms were the main factor regulating the N cycle. Second, amino acids in root exudates and the soil environment contributed 0.836 and 0.832 to the N cycle, respectively. Amino acids and soil SOC are the main nutrients for soil microbial activity. Amino acids can be directly utilized by microorganisms through cell membranes, while SOC needs to be decomposed by microorganisms before being utilized. Therefore, the contribution of amino acids to the N cycle is slightly higher than that of soil. The lowest contribution rate of soil enzyme activity was 0.640. The main function of urease is to promote the decomposition of urea; it decomposes urea into ammonium carbonate and then dissociates into ammonia (NH_3) and carbonic acid with the participation of water [76]. Therefore, the effect on the N cycle is related to the amount of urea applied. In the CK and straw treatments, the amount of urea was low or no urea was applied, so the effect on the N cycle was relatively low.

5. Conclusions

In this paper, long-term fertilization increased the TN content in the rhizosphere soil of maize, especially in the NPKS treatment, compared with that of CK, which increased by 0.34 g/kg. Fertilizer application increased the percentage of $\text{NH}_4^+ - \text{N}$ and $\text{NO}_3^- - \text{N}$ in TN and the emission of N_2O . Long-term fertilization had a significant effect on the soil enzyme activities involved in the N cycle. Protease and nitrifying enzymes increased significantly under long-term fertilization, while denitrifying enzymes decreased under long-term fertilization. Urease activity increased significantly in the straw addition treatment but decreased significantly in the N fertilizer application treatment. The functional genes of the N cycle in maize rhizosphere soil were mainly concentrated in the N degradation pathway, followed by the nitrogen fixation, denitrification, and ANRA pathways. The abundances of *nifA*, *ureC*, and *GDH2*; *amoA*, *amoB*, and *amoC*; and *nasD* of ANRA were decreased by applying N fertilizer, and the abundances of *nrfA* (DNRA) and *hao/hzo* (anaerobic ammonia oxidation and hydroxylamine oxidation) were increased by applying N fertilizer, which indicated that N fertilizer was the main factor regulating the N cycle. In addition, the contents of amino acids, organic acids, tyrosine, citric acid, and sucrose in the root exudates of maize were the highest, and the contents of the root exudates also changed significantly under long-term fertilization. The soil environment has a positive interaction with soil enzyme activity and a negative interaction with the microbial community composition and amino acids in root exudates in maize rhizosphere soil. Soil N cycling was influenced by soil, microorganisms, root exudates, and enzyme activity. The contribution rate of microorganisms to N cycling was the highest ($r^2 = 0.900$), followed by amino acids ($r^2 = 0.836$) and the soil environment ($r^2 = 0.832$). The contribution rate of soil enzyme activity was the lowest ($r^2 = 0.640$). Overall, our results highlight the importance of long-term fertilization in the N cycle and the interaction among plant, soil, and microbes in the plant–soil–microbe system that regulates the N cycle in the maize rhizosphere. However, this paper analyzed only the effects of plant–soil–microorganism interactions on the whole N cycle, and the effects on specific reaction processes need further analysis.

Author Contributions: Conceptualization, S.L., C.W. and Y.G.; methodology, C.W. and Y.Z.; software, Y.L. and J.W.; resources, S.L.; data curation, Y.L.; writing—original draft preparation, Y.L.; writing—review and editing, Y.L., Q.L. and C.W.; visualization, Y.G.; supervision, Q.L.; project administration, S.L.; funding acquisition, S.L. and Y.G. All authors have read and agreed to the published version of the manuscript.

Funding: This work received funding from the Natural Science Foundation of Jilin Province (20210101100JC) and the China Agriculture Research System of MOF and MARA (CARS-37).

Data Availability Statement: The data presented in this study are available in the articles.

Acknowledgments: We thank Hongyan Chang, Junhong Yao for soil collecting.

Conflicts of Interest: The authors declare no conflict of interest.

Appendix A

Table A1. Effect of long-term fertilizers on soil properties in maize rhizosphere.

	pH	SOC g/kg	DOC mg/kg	ROC g/kg	MBC mg/kg	TP g/kg	AP mg/kg	AK mg/kg	TK g/kg
CK	7.48 ± 0.06 a	18.77 ± 0.42 c	90.99 ± 6.20 c	3.92 ± 0.06 d	17.87 ± 0.06 a	6.20 ± 0.10 c	3.07 ± 0.38 c	211.69 ± 4.01 d	5.08 ± 0.15 a
N	6.37 ± 0.16 c	16.50 ± 0.27 d	95.54 ± 9.65 c	3.65 ± 0.19 d	16.47 ± 0.10 b	6.00 ± 0.08 cd	2.42 ± 0.41 c	156.37 ± 2.50 f	4.93 ± 0.46 a
NPK	6.77 ± 0.14 bc	18.68 ± 0.00 c	177.43 ± 5.79 b	4.95 ± 0.09 c	14.40 ± 0.16 c	9.92 ± 0.33 b	19.93 ± 2.14 b	187.70 ± 4.24 e	5.03 ± 0.59 a
S	6.97 ± 0.52 b	19.84 ± 0.31 b	181.54 ± 8.27 b	4.89 ± 0.06 c	14.32 ± 0.10 c	10.19 ± 0.29 b	23.40 ± 2.19 a	249.68 ± 3.07 c	5.15 ± 0.09 a
PKS	7.11 ± 0.10 ab	19.46 ± 0.20 b	195.62 ± 15.09 b	5.47 ± 0.14 b	12.48 ± 0.20 d	5.29 ± 0.97 d	4.20 ± 1.22 c	303.23 ± 8.91 b	5.05 ± 0.88 a
NPKS	7.17 ± 0.13 ab	21.28 ± 0.23 a	241.12 ± 17.55 a	6.22 ± 0.11 a	11.58 ± 0.15 e	11.27 ± 0.22 a	23.15 ± 0.80 a	325.39 ± 5.79 a	5.20 ± 0.05 a

Note: a total of 18 samples from 6 treatments; the lower case letters ‘a’, ‘b’, ‘c’, ‘d’, ‘e’, and ‘f’ indicate a significant difference ($p < 0.05$) among different samples for each treatment.

Table A2. Percentage of soil nitrogen on total nitrogen.

	NH ₄ ⁺ -N/TN %	NO ₃ ⁻ -N/TN %	ATN/TN %
CK	0.15	0.06	57.20
N	0.19	0.12	56.57
NPK	0.16	0.11	55.43
S	0.15	0.04	56.11
PKS	0.13	0.04	55.80
NPKS	0.15	0.04	57.20

Table A3. Correlation of principal components in principal component analysis.

	Composition				
	1	2	3	4	5
ROC	0.97	0.19	−0.06	0	
DOC	0.93	0.24	−0.11	0.07	
TN	0.92	0.17	−0.18	0.2	
SOC	0.89	−0.16	0.01	0.2	
NITs	0.89	−0.06			
Protease	0.88	−0.35			
Phenylalanine	0.81	0.08	−0.09	0.5	−0.28
Threonine	0.8	−0.32	0.49	−0.03	−0.08
Histidine	0.77	−0.41	−0.26	−0.1	0.35
Proline	0.77	−0.03	0.48	−0.38	−0.17
Arginine	−0.73	−0.08	0.55	0.36	0.05
Citric acid	−0.89	−0.33			
Oxalic acid	0.85	0.4			
Sucrose	0.9				
Elusimicrobia	0.96	0.06	0.06		
Nitrospirae	0.93	0.22	−0.21		
Firmicutes	0.89	0.13	−0.39		
Simpson	0.91				

Table A4. Correlation among soil, plant and bacteria.

		Bacteria					Soil				
		Simpson	Elusimicrobia	Nitrospirae	Firmicutes	NITs	Protease	SOC	DOC	ROC	TN
Plant	Phenylalanine	0.231	0.327	0.038	−0.438	−0.231	−0.162	−0.416	−0.365	−0.210	−0.533 *
	Threonine	0.430	0.281	0.380	0.011	−0.551 *	−0.637 **	−0.218	−0.564 *	−0.389	−0.639 **
	Histidine	−0.278	0.705 **	0.621 **	0.171	−0.768 **	−0.534 *	−0.696 **	−0.683 **	−0.654 **	−0.778 **
	Proline	0.181	−0.128	0.014	−0.168	−0.396	−0.164	0.231	−0.076	0.074	−0.161
	Arginine	0.458	−0.354	−0.189	0.1156	0.499 *	0.118	0.400	0.360	0.306	0.429
	Sucrose	−0.695 **	0.168	0.017	0.193	−0.115	0.346	−0.228	0.175	0.058	0.204
	Citric acid	0.684 **	0.141	0.218	−0.23	−0.131	−0.539 *	−0.207	−0.536 *	−0.404	−0.581 *
	Oxalic acid	−0.552 *	0.346	0.332	0.626 **	−0.357	−0.119	−0.252	−0.034	−0.079	0.047

Table A4. *Cont.*

		Bacteria						Soil		
		Simpson	Elusimicrobia	Nitrospirae	Firmicutes	NITs	Protease	SOC	DOC	TN
Soil	DOC	0.018	−0.740 **	−0.715 **	−0.294	0.620 **	0.877 **			
	ROC	0.172	−0.763 **	−0.749 **	−0.421	0.593 **	0.801 **			
	TN	−0.012	−0.754 **	−0.717 **	−0.250	0.655 **	0.832 **			
	SOC	0.306	−0.879 **	−0.646 **	−0.308	0.547 *	0.634 **			

Note: ‘*’ indicates a significant difference ($p < 0.05$) and ‘**’ indicates a significant difference ($p < 0.01$) among different samples for each treatment ($n = 18$) using the ANOVA method.

Table A5. Most contributing factor in each treatment to N cycle.

	r2	Pr (>r)
SOC	0.744	0.001
Methionine	0.820	0.001
Lactic Acid	0.737	0.001
Sucrose	0.744	0.001
Urease	0.706	0.001
Shannon	0.922	0.001
Actinobacteria	0.899	0.001

Table A6. Contribution of factors to N cycle.

	RDA1	RDA2	r2	Pr (>r)
bacterial composition	−0.638	0.770	0.900	0.001
bacterial diversity	−0.914	0.406	0.841	0.001
amino acids	0.100	−0.012	0.836	0.001
soil properties	−0.425	0.905	0.832	0.001
organic acids	−0.925	0.380	0.760	0.001
sugar	0.997	0.074	0.719	0.001
enzymes	−0.960	0.279	0.640	0.001

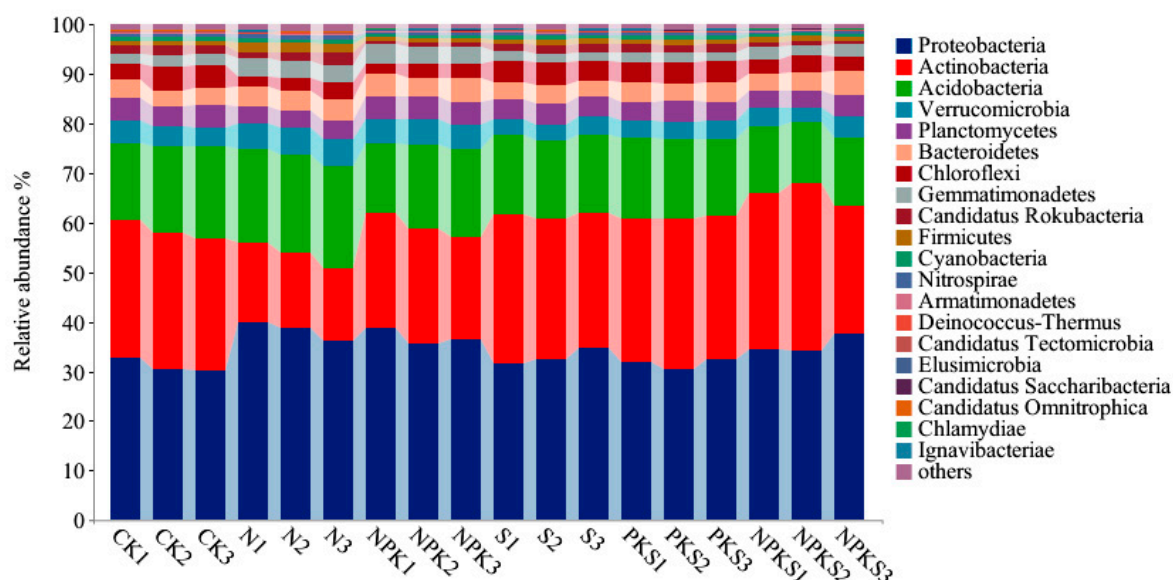


Figure A1. Relative abundance of bacterial community composition under long-term fertilization.

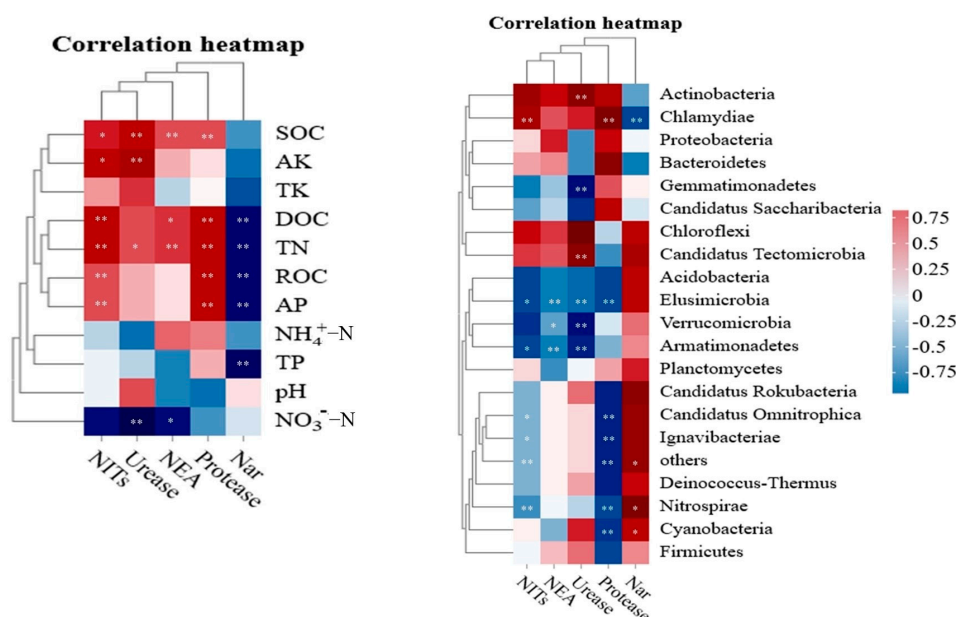


Figure A2. Correlation heatmap between soil enzyme activity and soil properties and bacteria. “*” indicates a significant difference ($p < 0.05$) and “**” indicates a significant difference ($p < 0.01$) between two variables for each treatment ($n = 18$) using Spearman’s rank correlation coefficients.

References

- Shah, T.; Lateef, S.; Noor, M.A. Carbon and Nitrogen Cycling in Agroecosystems: An Overview. In *Carbon Nitrogen Cycling in Soil*; Springer: Singapore, 2020; pp. 1–15.
- Xu, G.; Wang, Z.; Gao, M.; Tian, D.; Huang, R.; Liu, J.; Li, J. Effects of straw and biochar return in soil on soil aggregate and carbon sequestration. *Environ. Sci.* **2018**, *39*, 355–362.
- Liu, H.; Wu, M.; Gao, H.; Gao, J.; Wang, S. Application of (15) N tracing and bioinformatics for estimating microbial-mediated nitrogen cycle processes in oil-contaminated soils. *Environ. Res.* **2023**, *217*, 114799.
- Govednik, A.; Potočník, Ž.; Eler, K.; Mihelič, R.; Suhadolc, M. Combined effects of long-term tillage and fertilisation regimes on soil organic carbon, microbial biomass, and abundance of the total microbial communities and N-functional guilds. *Appl. Soil Ecol.* **2023**, *188*, 104876. [[CrossRef](#)]
- Sandén, T.; Spiegel, H.; Stüger, H.P.; Schlatter, N.; Haslmayr, H.P.; Zavattaro, L.; Grignani, C.; Bechini, L.; D’Hose, T.; Molendijk, L.; et al. European long-term field experiments: Knowledge gained about alternative management practices. *Soil Use Manag.* **2018**, *34*, 167–176. [[CrossRef](#)]
- Chen, D.; Liu, H.; Ning, Y.; Xu, C.; Zhang, H.; Lu, X.; Wang, J.; Xu, X.; Feng, Y.; Zhang, Y. Reduced nitrogen fertilization under flooded conditions cut down soil N_2O and CO_2 efflux: An incubation experiment. *J. Environ. Manag.* **2022**, *324*, 116335.
- Bento, C.B.; Brandani, C.B.; Filoso, S.; Martinelli, L.A.; Carmo, J.B.d. Effects of extensive-to-intensive pasture conversion on soil nitrogen availability and CO_2 and N_2O fluxes in a Brazilian oxisol. *Agric. Ecosyst. Environ.* **2021**, *321*, 107633.
- Dămățircă, C.; Moretti, B.; Bertora, C.; Ferrarini, A.; Lerda, C.; Mania, I.; Celi, L.; Gorra, R.; Zavattaro, L. Residue incorporation and organic fertilisation improve carbon and nitrogen turnover and stabilisation in maize monocropping. *Agric. Ecosyst. Environ.* **2023**, *342*, 108255.
- Ma, L.; Gao, W.; Luan, H.; Tang, J.; Li, M.; Huang, S. Effects of partial substitution of chemical fertilizer with manure and/or straw on the abundance of functional genes related to soil N-cycling. *J. Plant Nutr. Fertil.* **2021**, *27*, 1767–1778.
- Wang, D.; Yin, W.; Li, H.; Chen, L.; Zhao, P.; Long, G. Effects of intercropping and nitrogen application on soil microbial metabolic functional diversity in maize cropping soil. *Chin. J. Appl. Ecol.* **2022**, *33*, 793–800.
- Ye, L.F.; Liu, H.Y.; Dan Deng, H.; Zheng, Y.P.; Han, Y.W.; Gao, X.T.; Abbott, L.K.; Zhao, C.M.; Li, J.H. Effects of decadal nitrogen and phosphorus fertilization on microbial taxonomic and functional attributes associated with soil organic carbon decomposition and concentration in an alpine meadow. *Ecol. Indic.* **2023**, *146*, 109790.
- Leadbeater, D.R.; Oates, N.C.; Bennett, J.P.; Li, Y.; Dowle, A.A.; Taylor, J.D.; Alponi, J.S.; Setchfield, A.T.; Alessi, A.M.; Helgason, T. Mechanistic strategies of microbial communities regulating lignocellulose deconstruction in a UK salt marsh. *Microbiome* **2021**, *9*, 48. [[CrossRef](#)]
- Kuyper, M.M.M.; Marchant, H.K.; Kartal, B. The microbial nitrogen-cycling network. *Nat. Rev. Microbiol.* **2018**, *16*, 263–276. [[CrossRef](#)] [[PubMed](#)]
- Zhao, Y.; Li, Q.; Cui, Q.; Ni, S.-Q. Nitrogen recovery through fermentative dissimilatory nitrate reduction to ammonium (DNRA): Carbon source comparison and metabolic pathway. *Chem. Eng. J.* **2022**, *441*, 135938.

15. Chun, C.; Wu, Z.; Huang, Q.; Han, C.; Zhong, W. Effect of organic matter promotion on nitrogen-cycling genes and functional microorganisms in acidic red soil. *Environ. Sci.* **2020**, *41*, 2468–2475.
16. An, X.; Jiang, S.; Xie, C.; Xu, Y.; Xu, C.; Shen, Q. Effects of reducing chemical fertilizers combined with organic fertilizers on soil microbial community in litchi orchards. *Chin. J. Appl. Ecol.* **2022**, *33*, 1099–1108.
17. Tao, L. The Soil Microbial Activity and Microbial Functional Diversity of Cotton Field in Northern Xinjiang Response to Organic Fertilizer Partial Substitution for Chemical Fertilizer. Master's Thesis, Shihezi University, Shihezi, China, 2013.
18. Zhang, S. Effects of Chemical Fertilizers Partially Substituted by Organic Fertilizers on Potato Dry Matter Accumulation and Distribution and Soil Biological Characters. Ph.D. Thesis, Gansu Agricultural University, Lanzhou, China, 2014.
19. Castellano-Hinojosa, A.; Strauss, S.L.; González-López, J.; Bedmar, E.J. Changes in the diversity and predicted functional composition of the bulk and rhizosphere soil bacterial microbiomes of tomato and common bean after inorganic N-fertilization. *Rhizosphere* **2021**, *18*, 100362. [[CrossRef](#)]
20. Van Der Heijden, M.G.A.; Richard, D.B.; Van Straalen, N.M. The unseen majority: Soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. *Ecol. Lett.* **2008**, *11*, 296–310. [[CrossRef](#)]
21. Cui, J.; Zhu, R.; Wang, X.; Xu, X.; Ai, C.; He, P.; Liang, G.; Zhou, W.; Zhu, P. Effect of high soil C/N ratio and nitrogen limitation caused by the long-term combined organic-inorganic fertilization on the soil microbial community structure and its dominated SOC decomposition. *J. Environ. Manag.* **2022**, *303*, 114155. [[CrossRef](#)]
22. Enebe, M.C.; Babalola, O.O. Functional diversity of bacterial communities in the rhizosphere of maize grown on a soil under organic and inorganic fertilization. *Sci. Afr.* **2022**, *16*, e01212.
23. Liu, J.; Jin, L.; Shan, Y.; Burgess, K.S.; Ge, X. Elevation explains variation in soil microbial diversity and community composition under experimental warming and fertilization treatments in mountain meadows. *Appl. Soil Ecol.* **2022**, *171*, 104311. [[CrossRef](#)]
24. Das, P.P.; Singh, K.R.; Nagpure, G.; Mansoori, A.; Singh, R.P.; Ghazi, I.A.; Kumar, A.; Singh, J. Plant-soil-microbes: A tripartite interaction for nutrient acquisition and better plant growth for sustainable agricultural practices. *Environ. Res.* **2022**, *214*, 113821.
25. Fadji, A.E.; Yadav, A.N.; Santoyo, G.; Babalola, O.O. Understanding the plant-microbe interactions in environments exposed to abiotic stresses: An overview. *Microbiol. Res.* **2023**, *271*, 127368.
26. Pascale, A.; Proietti, S.; Pantelides, I.S.; Stringlis, I.A. Modulation of the Root Microbiome by Plant Molecules: The Basis for Targeted Disease Suppression and Plant Growth Promotion. *Front. Plant Sci.* **2019**, *10*, 1741. [[CrossRef](#)]
27. Li, W.; Li, Y.; Lv, J.; He, X.; Wang, J.; Teng, D.; Jiang, L.; Wang, H.; Lv, G. Rhizosphere effect alters the soil microbiome composition and C, N transformation in an arid ecosystem. *Appl. Soil Ecol.* **2022**, *170*, 104296. [[CrossRef](#)]
28. de Vries, F.T.; Griffiths, R.L.; Knight, C.G.; Niclitch, O.; Williams, A. Harnessing rhizosphere microbiomes for drought-resilient crop production. *Science* **2020**, *368*, 270–274. [[PubMed](#)]
29. Berendsen, R.L.; Pieterse, C.M.J.; Bakker, P.A.H.M. The rhizosphere microbiome and plant health. *Trends Plant Sci.* **2012**, *17*, 478–486. [[PubMed](#)]
30. Jacoby, R.; Peukert, M.; Succurro, A.; Koprivova, A.; Kopriva, S. The Role of Soil Microorganisms in Plant Mineral Nutrition—Current Knowledge and Future Directions. *Front. Plant Sci.* **2017**, *8*, 1617.
31. Wu, L.; Lin, X.; Lin, W. Advances and perspective in research on plant-soil-microbe interactions mediated by root exudates. *Chin. J. Plant Ecol.* **2014**, *38*, 298–310.
32. Ahmad, E.; Sharma, P.K.; Khan, M.S. Roles of Root Exudates in Different Processes in the Nitrogen Cycle in the Rhizosphere. *Soil Nitrogen Ecol.* **2021**, *62*, 179–200.
33. Wang, J.; Liao, L.; Wang, G.; Liu, H.; Wu, Y.; Liu, G.; Zhang, C. N-induced root exudates mediate the rhizosphere fungal assembly and affect species coexistence. *Sci. Total Environ.* **2022**, *804*, 150148.
34. Xu, Y. Effects of Simulated Root Secretions on the Abundance of Nitrification and Denitrification Genes. Master's Thesis, Anhui Jianzhu University, Hefei, China, 2018.
35. Yan, C.; Huang, J.; Li, Z.; Peng, C.; Cao, C. Effect of plant and its exudates on urease activity, nitrification and denitrification in wetland soil. *Ecol. Environ. Sci.* **2017**, *26*, 303–308.
36. Bian, X.; Zhao, W.; Yue, Z.; Wang, H.; Jiao, H.; Sui, H. Research process of soil enzymes on carbon and nitrogen cycle in agricultural ecosystem. *Chin. Agric. Sci. Bull.* **2016**, *32*, 171–178.
37. Li, Y.; Wang, C.; Chang, H.; Zhang, Y.; Liu, S.; He, W. Metagenomics reveals the effect of long-term fertilization on carbon cycle in the maize rhizosphere. *Front. Microbiol.* **2023**, *14*, 1170214.
38. Huang, R.; Lan, T.; Song, X.; Li, J.; Ling, J.; Deng, O.; Wang, C.; Gao, X.; Li, Q.; Tang, X.; et al. Soil labile organic carbon impacts C:N:P stoichiometry in urban park green spaces depending on vegetation types and time after planting. *Appl. Soil Ecol.* **2021**, *163*, 103926.
39. Xu, C.; Lin, X.; Xia, B. Response of root exudates of maize seedlings (*Zea mays* L.) to pyrene contamination. *Acta Ecol. Sin.* **2010**, *30*, 3280–3288.
40. Li, Y.; Wang, C.; Wang, T.; Liu, Y.; Jia, S.; Gao, Y.; Liu, S. Effects of Different Fertilizer Treatments on Rhizosphere Soil Microbiome Composition and Functions. *Land* **2020**, *9*, 329.
41. Yuan, X.; Niu, D.; Weber-Grullon, L.; Fu, H. Nitrogen deposition enhances plant-microbe interactions in a semiarid grassland: The role of soil physicochemical properties. *Geoderma* **2020**, *373*, 114446. [[CrossRef](#)]
42. Li, Q.; Jia, W.; Zhang, Q.; Cheng, X. Localized plant-soil-microbe interactions regulate spatial variations of soil oxidase activities within afforested systems in a subtropical area. *Geoderma* **2022**, *406*, 115499.

43. Ziadi, N.; Angers, D.A.; Gagnon, B.; Lalande, R.; Morel, C.; Rochette, P.; Chantigny, M.H. Long-term tillage and synthetic fertilization affect soil functioning and crop yields in a corn–soybean rotation in eastern Canada. *Can. J. Soil Sci.* **2014**, *94*, 365–376. [\[CrossRef\]](#)
44. Wang, X.; Liu, X.; Hao, L.; Xie, H.; Zhang, G.; Chen, Z.; Zhang, Y. Effects of straw returning in conjunction with different nitrogen fertilizer dosages on corn yield and soil properties. *Chin. J. Ecol.* **2020**, *39*, 507–516.
45. Sun, R.; Wang, D.; Lin, J.; Liu, Q.; Yang, L. Effects of long term integrated fertilization with organic manure and chemical fertilizers on soil nutrients in Taihu lake region. *Soils* **2009**, *41*, 384–388.
46. Chu, H.; Hosen, Y.; Yagi, K. NO, N₂O, CH₄ and CO₂ fluxes in winter barley field of Japanese Andisol as affected by N fertilizer management. *Soil Biol. Biochem.* **2007**, *39*, 330–339. [\[CrossRef\]](#)
47. Bei, S.; Zhang, Y.; Liu, J.; Mou, Y.; Lun, X. Greenhouse gas emission under the treatments of fertilization and wheat straw returning during the maize growing seasons. *Environ. Chem.* **2012**, *31*, 407–414.
48. Jian, S.; Li, J.; Chen, J.; Wang, G.; Mayes, M.A.; Dzantor, K.E.; Hui, D.; Luo, Y. Soil extracellular enzyme activities, soil carbon and nitrogen storage under nitrogen fertilization: A meta-analysis. *Soil Biol. Biochem.* **2016**, *101*, 32–43. [\[CrossRef\]](#)
49. Wang, J.; Fu, X.; Ghimire, R.; Sainju, U.M.; Jia, Y.; Zhao, F. Responses of soil bacterial community and enzyme activity to organic matter components under long-term fertilization on the Loess Plateau of China. *Appl. Soil Ecol.* **2021**, *166*, 103992. [\[CrossRef\]](#)
50. Miao, F.; Li, Y.; Cui, S.; Jagadamma, S.; Yang, G.; Zhang, Q. Soil extracellular enzyme activities under long-term fertilization management in the croplands of China: A meta-analysis. *Nutr. Cycl. Agroecosyst.* **2019**, *114*, 125–138.
51. Liu, H.; Lin, Y.H.; Zhang, Y.S.; Tan, X.X.; Wang, X.H. Effects of long-term fertilization on biodiversity and enzyme activity in grey desert soil. *Acta Ecol. Sin.* **2008**, *28*, 3898–3904.
52. Xue, D.; Yao, H.; He, Z.; Huang, C. Relationships between red soil enzyme activity and fertility. *Chin. J. Appl. Ecol.* **2005**, *16*, 1455–1458.
53. Shao, X.; Zheng, J. Soil Organic Carbon, Black Carbon, and Enzyme Activity Under Long-Term Fertilization. *J. Integr. Agric.* **2014**, *13*, 517–524.
54. Chang, N.; Zhai, Z.; Wang, L.; Deng, J. Impacts of nitrogen management and organic matter application on nitrous oxide emissions and soil organic carbon from spring maize fields in the North China Plain. *Soil Tillage Res.* **2020**, *196*, 104441.
55. Guo, J.; Wang, Y.; Lai, J.; Pan, C.; Zhang, L. Spatiotemporal distribution of nitrogen biogeochemical processes in the coastal regions of northern Beibu Gulf, South China Sea. *Chemosphere* **2019**, *239*, 124803. [\[CrossRef\]](#) [\[PubMed\]](#)
56. Liu, X.; Lee Burras, C.; Kravchenko, Y.S.; Duran, A.; Huffman, T.; Morris, H.; Studdert, G.; Zhang, X.; Cruse, R.M.; Yuan, X. Overview of Mollisols in the world: Distribution, land use and management. *Can. J. Soil Sci.* **2012**, *92*, 383–402. [\[CrossRef\]](#)
57. Liu, B.; Xia, H.; Jiang, C.; Riaz, M.; Yang, L.; Chen, Y.; Fan, X.; Xia, X. 14 year applications of chemical fertilizers and crop straw effects on soil labile organic carbon fractions, enzyme activities and microbial community in rice-wheat rotation of middle China. *Sci. Total Environ.* **2022**, *841*, 156608. [\[CrossRef\]](#)
58. Wang, R.; Wang, J.; Zhu, K.; Xiu, W.; Zhao, J.; Yang, D.; Li, G.; Liu, H. Abundance and diversity of diazotrophic nifH gene in the fluvo-aquic soil under different growth stages of maize. *J. Tianjin Norm. Univ. (Nat. Sci. Ed.)* **2018**, *38*, 35–41.
59. Leptin, A.; Whitehead, D.; Anderson, C.R.; Cameron, K.C.; Lehto, N.J. Increased soil nitrogen supply enhances root-derived available soil carbon leading to reduced potential nitrification activity. *Appl. Soil Ecol.* **2021**, *159*, 103842. [\[CrossRef\]](#)
60. Lyab, C.; Slabc, D.; Ygab, C.; Xia, X.; Jwab, C. Structure of rhizospheric microbial community and N cycling functional gene shifts with reduced N input in sugarcane-soybean intercropping in South China. *Agric. Ecosyst. Environ. Pollut.* **2021**, *314*, 107413.
61. Yang, L.; Muhammad, I.; Chi, Y.X.; Liu, Y.X.; Wang, G.Y.; Wang, Y.; Zhou, X.B. Straw return and nitrogen fertilization regulate soil greenhouse gas emissions and global warming potential in dual maize cropping system. *Sci. Total Environ.* **2022**, *853*, 158370. [\[CrossRef\]](#) [\[PubMed\]](#)
62. Hu, X.; Gu, H.; Liu, J.; Wei, D.; Zhu, P.; Cui, X.; Zhou, B.; Chen, X.; Jin, J.; Liu, X.; et al. Metagenomics reveals divergent functional profiles of soil carbon and nitrogen cycling under long-term addition of chemical and organic fertilizers in the black soil region. *Geoderma* **2022**, *418*, 115846.
63. Kelly, C.N.; Schwaner, G.W.; Cumming, J.R.; Driscoll, T.P. Metagenomic reconstruction of nitrogen and carbon cycling pathways in forest soil: Influence of different hardwood tree species. *Soil Biol. Biochem.* **2021**, *156*, 108226.
64. Moormann, J.; Heinemann, B.; Hildebrandt, T.M. News about amino acid metabolism in plant–microbe interactions. *Trends Biochem. Sci.* **2022**, *47*, 839–850. [\[CrossRef\]](#)
65. Xiao, W.; Zhang, Q.; Zhao, S.; Chen, D.; Gao, N.; Huang, M.; Ye, X. Citric acid secretion from rice roots contributes to reduction and immobilization of Cr(VI) by driving microbial sulfur and iron cycle in paddy soil. *Sci. Total Environ.* **2023**, *854*, 158832. [\[CrossRef\]](#) [\[PubMed\]](#)
66. Yoon, J.; Cho, L.-H.; Tun, W.; Jeon, J.-S.; An, G. Sucrose signaling in higher plants. *Plant Sci.* **2021**, *302*, 110703. [\[CrossRef\]](#) [\[PubMed\]](#)
67. Li, Y.; Lv, Y.; Lian, M.; Peng, F.; Xiao, Y. Effects of combined glycine and urea fertilizer application on the photosynthesis, sucrose metabolism, and fruit development of peach. *Sci. Hortic.* **2021**, *289*, 110504. [\[CrossRef\]](#)
68. Tan, M.; Zong, R.; Lin, H.; Dhital, Y.P.; Ayantobo, O.O.; Chen, P.; Li, H.; Chen, R.; Wang, Z. Responses of soil nutrient and enzyme activities to long-term mulched drip irrigation (MDI) after the conversion of wasteland to cropland. *Appl. Soil Ecol.* **2023**, *190*, 104976. [\[CrossRef\]](#)

69. Wang, Z.; Wang, K.; Jiang, Q.; Liu, C.; Shan, J.; Teng, H. Mutual feeding mechanism of carbon, nitrogen and enzyme activity in Northeast China black soil under snow cover change. *Appl. Soil Ecol.* **2023**, *190*, 104991. [[CrossRef](#)]
70. Sasse, J.; Martinoia, E.; Northen, T. Feed Your Friends: Do Plant Exudates Shape the Root Microbiome? *Trends Plant Sci.* **2018**, *23*, 25–41. [[CrossRef](#)]
71. Wen, T.; Yu, G.-H.; Hong, W.-D.; Yuan, J.; Niu, G.-Q.; Xie, P.-H.; Sun, F.-S.; Guo, L.-D.; Kuzyakov, Y.; Shen, Q.-R. Root exudate chemistry affects soil carbon mobilization via microbial community reassembly. *Fundam. Res.* **2022**, *2*, 697–707. [[CrossRef](#)]
72. Maurer, D.; Malique, F.; Alfarraj, S.; Albasher, G.; Horn, M.A.; Butterbach-Bahl, K.; Dannenmann, M.; Rennenberg, H. Interactive regulation of root exudation and rhizosphere denitrification by plant metabolite content and soil properties. *Plant Soil* **2021**, *467*, 107–127. [[CrossRef](#)]
73. Wang, H.; Zheng, T.; Yang, X. Molecular ecology research progress for soil denitrification and research status for its influencing factors. *J. Agro-Environ. Sci.* **2013**, *32*, 1915–1924.
74. Liu, Y.; Chen, J.; Liu, Q.; Chen, L. Advances in studies of soil nitrification and denitrification and controlling factors. *J. Sichuan For. Sci. Technol.* **2006**, *27*, 36–41.
75. Carrara, J.E.; Walter, C.A.; Hawkins, J.S.; Peterjohn, W.T.; Averill, C.; Brzostek, E.R. Interactions among plants, bacteria, and fungi reduce extracellular enzyme activities under long-term N fertilization. *Glob. Chang. Biol.* **2018**, *24*, 2721–2734. [[CrossRef](#)] [[PubMed](#)]
76. Jiao, X.; Gao, C.; Lü, G.; Sui, Y. Effect of Long-Term Fertilization on Soil Enzyme Activities under Different Hydrothermal Conditions in Northeast China. *Agric. Sci. China* **2011**, *10*, 412–422. [[CrossRef](#)]

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.